Remarks

By the present invention there are provided methods for detecting the presence of oligonucleotides and monitoring oligonucleotide production during amplification. The methods use fluorescence energy transfer by competitive hybridization, wherein competitive hybridization occurs between a fluorophore labeled first probe and target nucleic acid, and between the first probe and a quencher labeled second probe. Fluorescence signal due to the first probe fluorophore increases as target nucleic acid competes away the first probe from binding to and being quenched by the second probe.

Claims 2-11 and 14-22 are pending and under active prosecution. The **Listing of Claims** with appropriate status identifier begins on page 2 of this communication. Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

By the present communication, Claim 20 has been amended to define Applicants' invention with greater particularity. No new matter has been introduced as the subject amendment is fully supported by the specification and claims as originally filed. Specifically, the present invention provides methods of real-time detection of PCR product during PCR. See Example 3, Specification, page 9, lines 12-26 and Figure 1. Indeed, in Example 3, the invention method is conducted in the same tube as used for PCR. See Specification, page 9, line 20. Additionally, Figure 1 presents results (i.e., fluorescence enhancement due to product accumulation) during an actual PCR experiment. Accordingly, the present amendment to Claim 20 is made to clarify that the present invention is used to monitor the target nucleic acid during amplification.

Additionally, an amendment to Claim 20 is made to delete "can" used in reference to hybridization. This amendment raises no issue of new matter.

Rejection under 35 U.S.C. § 112, second paragraph

The rejection of Claims 2-11 and 13-22 under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness with respect to the phrase "can hybridize" is respectfully traversed. Although Applicant does not agree that the term "can" is indefinite, the term has been deleted from the claims to expedite prosecution. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Rejection under 35 U.S.C. § 102

The rejection of Claims 3-6, 8-10, 20 and 22 under 35 U.S.C. § 102(e) as allegedly being anticipated by Heller et al. (U.S. 5,849,489) is respectfully traversed.

In order to anticipate a claim, a single prior art reference must provide each and every element set forth in the claim. *In re Bond*, 15 USPQ2d 1566, 1567 (Fed. Cir. 1990). *See also*, MPEP §2131. The Examiner bears the initial burden of establishing a *prima facie* case of anticipation. Only when a *prima facie* case has been established does the burden shift to the applicant to rebut the *prima facie* case. *See, e.g., In re Morris*, 127 F.3d 1048, 1054 (Fed. Cir. 1997). For the reasons that follow, it is respectfully submitted that Heller does not describe each and every element of the claimed subject matter.

The Examiner has apparently misapprehended the invention of Heller to have a broader scope than is reasonably described or enabled therein. Whereas the present invention provides for monitoring of nucleic acid amplification during amplification, contrary to the Examiner's apparent misconception, Heller describes a static (i.e., non-cycling) assay. Indeed, Heller provides no description or enablement whatsoever for real-time monitoring of amplified nucleic acids. Specifically, Heller describes no polymerase, no mention of deoxynucleotide triphosphates, no mention to use a cycling mechanism characteristic of PCR, and no mention real-time acquisition of signal during multiple amplification steps.

What Heller does describe is a photonic structure comprising a plurality of donor chromophores and methods and uses thereof:

The present invention contemplates chromophore-containing polynucleotides having at least two donor chromophores operatively linked to the polynucleotide by linker arms, ... to form a photonic structure for collecting photonic energy and transferring the energy to an acceptor chromophore, and methods using the photonic structures. (Abstract)

The "collecting photonic energy" element is elaborated in the Specification (Column 4, lines 59-65) which clarifies that:

The present invention therefore describes a polynucleotide having at least two (multiple) donor chromophores operatively linked to the polynucleotide by linker arms, such that the chromophores are positioned by the linkage along the length of the polynucleotide at a donor--donor transfer distance. Typically the donor chromophores are nonfluorescing chromophores.

Further examples of the photonic structures and uses thereof of Heller are found throughout the specification, including use in a diagnostic assay system that contains a polynucleotide capable of donor-donor transfer (Column 5, lines 16-17), and nucleic acid duplex structures comprising at least two hybridized polynucleotides which contain operatively linked donor and acceptor chromophores to provide a larger structure upon which donor-donor and donor-acceptor energy transfer can occur (Column 5, lines 331-39). In all such passages, Heller describes no polymerase, no mention of deoxynucleotide triphosphates, no mention to use a cycling mechanism characteristic of PCR, and no mention real-time acquisition of signal during multiple amplification steps.

In further examples of the methods of Heller, the Specification describes (Column 19, lines 8-40) a diagnostic method including the steps of:

a) admixing:

(i) a polynucleotide having (1) at least two donor chromophores operatively linked to a polynucleotide by linker arms, such that the chromophores are positioned by linkage along the length of the polynucleotide at a donor--donor transfer distance, and (2) at least one fluorescing acceptor chromophore operatively linked to the polynucleotide by a linker arm, such that the fluorescing acceptor chromophore is positioned by linkage at a donor-acceptor transfer distance from at least one of the donor chromophores, wherein the polynucleotide has a nucleotide sequence that is preselected as to be complementary to the preselected "target" nucleic acid sequence; with

(ii) a nucleic acid-containing sample containing the preselected nucleic acid base ("target") sequence to form a hybridization reaction admixture;
(b) subjecting the hybridization reaction admixture to hybridization conditions for a time period sufficient for the polynucleotide to hybridize to the target sequence and form a donor chromophore containing- and acceptor chromophore containing-hybridized nucleic acid duplex;
(c) exciting the donor chromophore in the nucleic acid duplex formed in step (b) by exposing the donor chromophore to sufficient photonic energy to induce emission of photonic energy from the acceptor chromophore; and
(d) detecting the presence of photonic energy emitted from the excited acceptor chromophore, thereby detecting the presence of the preselected nucleic acid sequence in the sample.

In the above detailed methodology, Heller describes no polymerase, no mention of deoxynucleotide triphosphates, no mention to use a cycling mechanism characteristic of PCR, and no mention real-time acquisition of signal during multiple amplification steps.

In a further elaboration of the above method described in Column 19, lines 53-58, the incorporation of a quencher is described:

In another embodiment, the hybridization admixture can contain a quencher polypeptide as described herein, having a nucleic acid sequence designed to compete with the target sequence for hybridization with the polynucleotide containing the target nucleic acid sequence. The embodiment is shown in Example 3 and FIG. 4.

Again, Heller describes no polymerase, no mention of deoxynucleotide triphosphates, no mention to use a cycling mechanism characteristic of PCR, and no mention real-time acquisition of signal during multiple amplification steps.

In a further elaboration of homogeneous hybridization reactions useful for the above method, the Examiner's attention is drawn to the only reference within Heller which mentions PCR (Column 21, lines 13-20):

Also preferred are the homogeneous hybridization reactions such as are conducted for a reverse transcription of isolated MRNA to form cDNA, dideoxy sequencing and other procedures using primer extension reactions in which polynucleotide hybridization is a first step. Particularly preferred is the homogeneous hybridization reaction in which a specific nucleic acid sequence is amplified via a polymerase chain reaction (PCR).

It is respectfully submitted to be clear from the context of this passage that Heller at most only describes preferred hybridization conditions (i.e., buffer, temperature, etc.) for the above static assay method. This passage is typical of the entire Heller patent in being silent as to the use in the assay of a polymerase, deoxynucleotide triphosphates, a cycling mechanism characteristic of PCR, or use of real-time acquisition of signal during multiple amplification steps.

In summary, Heller does not describe, enable, or contemplate a method for monitoring of target nucleic acid during amplification. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 103

To establish a *prima facie* case of obviousness, three criteria must be met: there must be some motivation or suggestion, either in the cited publications or in knowledge available to one skilled in the art, to modify or combine the cited publications; there must be a reasonable expectation of success in combining the publications to achieve the claimed invention; and the publications must teach or suggest all of the claim limitations. See MPEP §2142. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 493; 20 USPQ2d 1438, 1442 (Fed. Cir. 1991); see also MPEP §2142. In analyzing obviousness, the Court of Appeals for the Federal Circuit has repeatedly cautioned that:

> [t]he factual inquiry... must be based upon objective evidence of record.... [T]he best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references.... [P]articular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

In re Sang-*Su Lee*, 277 F.3d 1338, 1343 (Fed. Cir. 2002), 61 USPQ2d 1430, 1433 (internal citations omitted).

The 103(a) Rejection Over Heller in view of Morrison

The rejection of Claims 2, 11 and 21 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Heller et al. as applied to claims 3-6, 8-10, 20 and 22 further in view of Morrison et al. (*Anal. Biochem.* 1989, **183**: 231-244) is respectfully traversed. As acknowledged by the Examiner, Heller does not disclose a thermostable polymerase. In an attempt to find a motivation to combine, the Examiner asserts that Morrison describes a thermostable polymerase.

Applicants respectfully submit that no *prima facie* rejection has been stated because Morrison fails to cure the defects of Heller as discussed under the § 102(e) rejection above. Specifically, Morrison, like that of Heller, describes a static assay for detecting a nucleic acid. For example, Morrison describes (page 235, first paragraph) assays conducted on the results of a PCR amplification experiment wherein the samples were stored for subsequent analysis, and wherein each assay required half of the stored sample. It is respectfully submitted that such use does not teach or suggest the claimed method of amplifying a nucleic acid and monitoring it during the amplification. Morrison, like that of Heller, fails to describe use of a polymerase, deoxynucleotide triphosphates, use of a cycling mechanism characteristic of PCR, and use of real-time acquisition of signal during multiple amplification. As such, the rejection fails to teach or suggest all of the claim limitations. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

The 103(a) Rejection Over Heller in view of Hiroaki

The rejection of Claims 14-18 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Heller et al. as applied to claims 3-6, 8-10, 20 and 22 further in view of Hiroaki et al (EP 0461 863 A1) is respectfully traversed. As acknowledged by the Examiner, Heller does not disclose that the target polynucleotide comprises hepatitis C virus genome, the probe having the sequence of SEQ ID NO: 3 and 4 and the primer having the sequence of SEQ ID NO: 1 and 2. In an attempt to find a motivation to combine, the Examiner asserts that Hiroaki discloses a nucleotide which comprises SEQ ID NO:1 and 3 and the complementary sequence of SEQ ID

NO: 2 and base pair 1-17 of SEQ ID NO: 4 (Office Action dated May 22, 2006, page 5, paragraph 4, lines 6-8).

Applicants respectfully submit that no *prima facie* rejection has been stated because Hiroaki fails to cure the defects in the teachings of Heller as discussed under the § 102(e) rejection above. Specifically, Hiroaki makes no reference whatsoever to monitoring of amplified nucleic acid during amplification. As such, the rejection fails to teach or suggest all of the claim limitations. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

The 103(a) Rejection Over Heller in view of Meade

The rejection of Claim 7 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Heller et al. as applied to claims 3-6, 8-10, 20 and 22 further in view of Meade et al (US 5,824,473) is respectfully traversed. As acknowledged by the Examiner, Heller does not disclose a fluorophore being on the 3' terminal of the first probe and the quencher being on the 5' terminal of the second probe. In an attempt to find a motivation to combine, the Examiner asserts that Meade discloses the selective modification of nucleic acid at specific sites with redox active moieties, which moieties can be switched either on 5' or 3' terminal (Office Action dated May 22, 2006, page 6, paragraph 4, lines 1-3).

Applicants respectfully submit that no *prima facie* rejection has been stated because Meade fails to cure the defects in the teachings of Heller as discussed under the § 102(e) rejection above. Specifically, Meade makes no reference whatsoever to monitoring of amplified nucleic acid during amplification. As such, the rejection fails to teach or suggest all of the claim limitations. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

The 103(a) Rejection Over Heller in view of Walker

The rejection of Claim 19 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Heller et al. as applied to claims 3-6, 8-10, 20 and 22 further in view of Walker et al (US 5,270,184) is respectfully traversed. As acknowledged by the Examiner, Heller does not disclose a strand displacement amplification method. In an attempt to find a motivation to combine, the Examiner asserts that Walker discloses strand displacement amplification for generating target nucleic acid sequence (Office Action dated May 22, 2006, page 7, paragraph 3, lines 1-2).

Applicants respectfully submit that no *prima facie* rejection has been stated because Walker fails to cure the defects in the teachings of Heller as discussed under the § 102(e) rejection above. Specifically, Walker makes no reference whatsoever to monitoring of amplified nucleic acid during amplification, describing instead only static diagnostic assays for example with beads, membranes or solid supports (Column 5, lines 1 1-15) or homogeneous systems including for example fluorescence polarization, enzyme mediated immunoassays, fluorescence energy transfer, hybridization protection (e.g., acridinium luminescence) and cloned enzyme donor immunoassays (Column 12, lines 49-59). Walker does not teach or suggest at least the missing claim element (i.e., monitoring during amplification) which renders Heller defective. As such, the rejection fails to teach or suggest all of the claim limitations. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Conclusion

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. In the event that any matters remain to be solved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

No fee is believe due with the present communication. However, the Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872.

Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

Date 07/17/2006

By _____

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